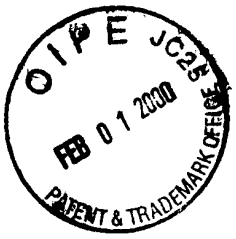
2/3/1/00 #4

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE



In re Application of:

PRAVEEN SHARMA et al

Appln. No.: **09/429,003**

Filed: **October 29, 1999**

55

Group Art Unit: **1649**

Examiner: **Unknown**

For: **METHOD OF PREPARING A STANDARD DIAGNOSTIC GENE TRANSCRIPT PATTERN**

SUBMISSION OF PRIORITY DOCUMENT

Assistant Commissioner
for Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith is a certified copy of the original priority document (NO 972006) on which claim to priority was made under 35 U.S.C. § 119.

The Examiner is respectfully requested to acknowledge receipt of said priority document.

Respectfully submitted,

Gordon Kit
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Date: February 1, 2000

KONGERIKET NORGE
The Kingdom of Norway

Bekreftelse på patentsøknad nr
Certification of patent application no

1997 2006

- Det bekreftes herved at vedheftede dokument er nøyaktig utskrift/kopi av ovennevnte søknad, som opprinnelig inngitt 1997.04.30

- *It is hereby certified that the annexed document is a true copy of the above-mentioned application, as originally filed on 1997.04.30*

2000.01.06

Freddy Strømmen
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PATENTSTYRET
Styret for det industrielle rettsvern

BL. - 130 - 5000 - 8.98 - Lørenskog Trykk as

Søknad om patent.

Søknadsskriv

1a - d

Styret for det industrielle rettsvern

Dato	Patentsøknad nr.
30. APR 97	972006

Til
Patentstyret
Boks 8160 Dep.
0033 Oslo

Utfylles av Styret

Patentsøknad nr.
Inngivelsesdag
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Behandlende medlem UF
Int. Cl.[6] C12Q

Søkers/fullmektigs referanse (angis hvis ønsket):

Alm.tilgj - 2 NOV. 1998

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Oppfinnelsens benevnelse:

Hvis søknaden er en internasjonal søknad som videreføres etter patentlovens § 31:

Den internasjonale søknads nummer .....

Den internasjonale søknads inngivelsesdag .....

Søker:
Navn, bopel og adresse.
(Hvis patent søkes av flere. Opplysning om hvem som skal være bemyndiget til å motta meddelelser fra Styret på vegne av søkerne).
(Fortsett om nødvendig på neste side)

Forskningsparken i Ås AS, Sagabygget, N-1432 Ås.

Oppfinner:
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Fullmektig:

Hvis søknad tidligere er inngitt i eller utenfor riket:
(Fortsett om nødvendig på neste side)

Prioritet kreves fra dato..... sted ..... nr. .....

Prioritet kreves fra dato..... sted ..... nr. .....

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Hvis avdelt søknad:

Den opprinnelige søknads nr.:.....og dennes inngivelsesdag .....

Hvis utskilt søknad:

Den opprinnelige søknads nr.:..... begjært inngivelsesdag.....

972006

Angivelse av tegnings-figur som ønskes publisert sammen med sammendraget

Fig. nr. .....

POSTADRESSE
Boks 8160 Dep.
0033 Oslo

KONTORADRESSE
Københavngt. 10
Oslo

TELEFON
22 38 73 00

TELEKS
19 152
nopat n

TELEFAKS
22 38 73 01

POSTGIRO
0808 5170709

BANKGIRO
1600.40.39916

Bl. 10 - 10 000. 1-97. Sem Grafisk

FORSKNINGSPARKEN I ÅS AS
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Date: 28.04.1997

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PATENT APPLICATION:

NOVEL METHOD FOR THE DIAGNOSIS OF DISEASE AND MALAISE

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SUMMARY:

In order to identify diseases, malaises or other conditions caused by other organisms, toxins, stress, aging etc. in human beings, animals, plants and all other living eukaryotic organisms, a set of diagnostic probes may be designed determining the activity of genes, as a standard for each relevant condition, relative to which a selective set of transcripts or mRNA may be measured based on a sample of tissue or body fluid removed from the live organism to be diagnosed.

NOVEL METHOD FOR THE DIAGNOSIS OF DISEASE AND MALAISE

This invention relates to both a diagnostic principle and a method of identifying diseases, malaises and syndromes in any eukaryotic organism as well as the associated method for the design and development of diagnostic probes to be used in the relative measurements necessary to reach specific diagnosis or to identify relevant conditions.

The invention shows a quick and precise method for the diagnosis of any disease or condition that leads to alterations in the activity of genes in a pattern which is specific to any particular condition of the organism under observation.

From the very early stages of diseases caused by infections, toxic substances, ageing or other conditions changing the quality of life of living eukaryotic organisms, the whole organism responds to the changed condition. This respons occurs, throughout the organism, even if only a minor part of the organism appears to be affected. The response lasts until the condition is healed or until the death of the affected organism.

Usually the reactions to infections, toxins or deteriorations are accompanied by changes in the level of activity in several or many genes. These activity levels, that may either be relatively higher or lower, are specific to the type of condition that is encountered. The normal activity and the altered activity may, to a large extent, be measured by the amount of specific transcripts or mRNA that is present. Thus standardised probes for analysis may be designed that have patterns of activity that are are characteristic for each condition or combination of conditions that is to be identified or diagnosed. These standardised probes may be used to compare the standardised probe pattern with transcript patterns from samples of tissue or body fluids prepared in a similar way and obtained from a live patient or the organism to be studied.

There are numerous examples of diagnostic methods that span from physical, anatomical and behavioural examination as well as biochemical, electric, electromagnetic means of supplementing the observation of the patient.

These diagnostic methods are well developed and are often efficient means to identify many pathological conditions. They are based on resent development and research as well as on the transfer of observations, experience and empirical data recorded by health-workers concerned with diseases of human beings, other animals and plants for at least 6000 years.

Never before has the arsenal of diagnostic tools been greater than at present, but even so, incorrect diagnosis of ailments and other conditions are still commonplace.

New diseases and conditions are found that may be related to environmental changes or mutations or other alterations in both the active agents or organisms as well as in the organism that is exposed. In addition a number of old and new illegal substances used in sports and by drug addicts can be included.

Several conditions are not easily identified with the available methods and/or the conclusive identification of a disease or condition may be reached too late for adequate corrective treatment.

Even if a great number of differential diagnostic methods have been developed, there is still a considerable number of closely related conditions, or combinations of conditions that resist quick safe and sure identification at low cost.

Furthermore, a number of diagnostic methods depend on the injection of foreign fluids or other kinds of transfer of diagnostic aids onto or into the organism under observation, or require biopsies. The removal of sample tissue from parts of the organisms often not easily accessible may also have a detrimental effect on the identification process and healing process itself.

Due to the extensive time often encountered in a complete diagnostic procedure, incorrect antibiotic therapy is often started prematurely, before a conclusive diagnosis is reached. This medical practice can aggravate the serious development of bacterial strains resistant to antibiotics.

The ability to design diagnostic standard probes for the identification of traditional conditions, that at present are hard to identify, as well as quickly to adapt the design of new probes for the identification of new conditions that may appear, as soon as they are identified, will therefore be of great value.

The advantages of the invention are of both primary and secondary nature:

Samples of tissue or body fluids may be obtained from parts of the organism that is not affected by the condition under observation.

Only one sample will suffice for a complete identification, thus great reductions in costs, time and inconvenience may result by avoiding hospitalization during the normal extensive range of diagnostic tests performed on human and other animal patients.

No foreign substances need be introduced onto or into the organism under observation in order to aid in the identification of the condition, thus the invention will reduce the risk of anaphylactic reactions to such induced diagnostic substances.

The invention has the potential to detect most diseases and syndromes of somatic, psycho-somatic and mental character as well as detecting deterioration due to ageing of the organism. In addition the method will detect the organism's reactions to toxic substances, radiation, pesticides, antibiotics, drugs, allergens and combinations of several such conditions.

The invention will furthermore make it possible to detect diseases or undesirable conditions in an organism at very early stages, even years before other subjective or objective symptoms may appear.

Even in cases where the patient dies from a hitherto not identified condition, and the cause of death is not established until a forensic post-mortem examination has been performed, the principle will be of value. If, in the attempts to diagnose the patient prior to death, a series of patient specific probe patterns were developed, these probes may be used for the design of new standard probe patterns that may be used to diagnose later occurrences of similar conditions.

The analytical instruments and equipment necessary to make use of the invention is readily available in laboratories engaged in standard biochemical and bio-technological work.

To describe the invented principle and method as clearly as possible, the word "patient" is used, even if the principle and method applies equally to all eukaryotic organisms. Furthermore "ailment" is used, even if the principle and method applies equally to any condition that leads to a change of activity in the informative genes of any eukaryotic organism.

In order to put the invention into practical use, two kinds of substantially similarly developed diagnostic probes must be available for comparison.

1- A Standard Diagnostic Probe Pattern (SDPP) that is characteristic of the suspected ailment, developed from one or several previously clearly diagnosed patients.

and

2- A Patient-Specific Probe Pattern (PSPP) that is developed from a resently obtained sample of tissue or body fluid from a patient.

To apply the invention to a specific condition, the pattern of a SDPP characteristic of the suspected ailment must have been developed on beforehand. In addition, a resent, well preserved sample of tissue or body fluid from the patient must be available to develop the PSPP, for comparison with one or several different SDPPs for the number of ailments and their different stages that is suspected.

To design and develop the pattern for SDPPs, characteristic for one ailment, known techniques of isolation of mRNA, construction and amplification of cDNA and selection through differential hybridisation and differential display may be used.

Selected informative cDNA probes from one or more patients that have been conclusively diagnosed with the ailment in question are isolated and amplified. These SDPPs together will be used to compare if the PSPP is similar to the SDPP. Several such characteristic SDPPs may be developed to represent different stages of the same ailment.

The pattern of such standard probes for a great number of ailments and different stages of such ailments may be accumulated in data bases and be made available to laboratories on request.

Examples of the procedure for concrete diagnosis of an ailment may best be described by the following examples.

Example 1: Diagnosis of Alzheimer syndrome

- A blood-sample is collected from a patient suspected of suffering from the ailment.
- The sample is immediately preserved in liquid nitrogen to prevent degradation of the mRNA of the sample.

- The sample is transferred for analysis, the mRNA is converted to cDNA, amplified through PCR, (Polymerase Chain Reaction) and labelled with suitable radioactive nucleotides.

- The labelled cDNA is hybridized ~~to~~ to several diagnostic DNA probes that are immobilised on a filter.
-
The radioactive signals from the (~~PBS~~ PSPP) filter is quantified using an Instant Imager and the relative signal value from each of the different probes is determined.

- The relative values from the different probes will together create a pattern that is specific for the patients ailment, the PSPP.

- This specific pattern for the patient is compared to the SDPPs characteristic of Alzheimer syndrome in different stages, if the patterns of the SDPP and the PSPP coincide, the ailment and its stage is diagnosed with great certainty.

If the patterns do not coincide, the ailment may be ruled out and the same PSPP may be compared manually or automatically to any number of available SDPPs until a match is found.

Example 2: Diagnosis of senile dementia

The patient specific sample is collected, preserved, the mRNA is converted to cDNA, amplified and labelled through the same procedure as described in example 1.
- The comparative hybridization is performed with a different set of SDPPs that have been developed to distinguish between different kinds and stages of senile dementia.

Example 3: Broad spectrum health status
The patient specific sample is collected, preserved, the mRNA is converted to cDNA, amplified and labelled through the same procedure as described in example 1.

- Primers to detect a set of transcripts are used for labelling.

- The labelled samples are separated through gel-electrophoresis and all the DNA fragments are detected and quantified.

- The resulting pattern specific to the patient PSPP is subsequently compared to SDPPs of a number of different diseases. For every mismatch encountered the associated ailment may be ruled out.

CLAIMS

Claim 1 A principle and method of diagnosing ailments or conditions of patients or eukaryotic organisms by the design of and development of standard probe patterns of the amount of transcript from two or more informative genes relative to a standard, each such standard probe pattern being characteristic of one ailment and/or stage of such ailment, said standard probe patterns ARE SUBSEQUENTLY compared with a pattern of transcript levels, using the same probes, prepared from a resent sample of tissue or body fluid collected from a patient to be diagnosed, such patterns being specific to the present condition of the patient.

Claim 2 A principle and method according to claim 1 characterised by the said transcripts, that when measured, may be in the form of RNA or altered to modified forms of RNA and/or into modified or unmodified forms of DNA and/or in the form of, primers, antibodies or other molecules that may enable specific hybridization into said forms of RNA, DNA or combinations of these.

Claim 3 A principle and method according to claim 1 characterised by that the said standard, may be based on a transcript, a combination of transcripts, the total amount of transcripts or total amount of RNA or representative forms of these alternatives or other forms of and combinations of cellular molecules.

Claim 4 A principle and method according to claim 1 characterised by that the said ailments applies to all conditions, ailments, diseases or reactions that leads to the relative increase or decrease in the activity of informative genes of any or all eukaryotic organisms regardless of these changes being caused by the influence of bacteria, virus, prions, parasites, fungi, radiation, natural or artificial toxins, drugs or allergens, furthermore including mental conditions due to stress, neurosis, psychosis or deteriorations due to the ageing of the patient or organism.

Claim 5 A principle and a method according to claim 1 characterised by that the said word patient applies to all eukaryotic organisms as human beings, other mammals and animals, birds, insects, fish and plants.